PATENT COOPERATION TREATY

RECEIVED
20 OCT 2004

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

Franks, Barry
AMERSHAM PLC
The Grove Centre
White Lion Road
Amersham, Buckinghamshire HP7 9LLDB:
GRANDE BRETAGNE

DUE DATE:	—
FORMALITIES:	HSL ✓
PAT. OFF:	MRB
DB:	—
CASE NO:	PA0247-PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*: 20 OCT 2004

Applicant's or agent's file reference: PA0247-PCT

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 02/03142	08.07.2002	08.07.2002

Applicant: AMERSHAM BIOSCIENCES UK LIMITED et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized Officer

Lafitte-de Jong, S

Tel. +31 70 340-4827

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PA0247-PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 02/03142	International filing date *(day/month/year)* 08.07.2002	Priority date *(day/month/year)* 08.07.2002
International Patent Classification (IPC) or both national classification and IPC G01N33/68		
Applicant AMERSHAM BIOSCIENCES UK LIMITED et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23.01.2004	20 OCT 2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Ginoux, C Telephone No. +31 70 340-2839

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 02/03142

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-44	as originally filed

Claims, Numbers

1-6, 7 (part)	as originally filed
7 (part), 8-19	received on 07.07.2004 with letter of 06.07.2004

Drawings, Sheets

1/2-2/2	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-7,15-19
	No:	Claims	8-14
Inventive step (IS)	Yes:	Claims	
	No:	Claims	8-14
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	

2. Citations and explanations

see separate sheet

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
Reference is made to the following document:

D1: ERNST L A ET AL: 'CYANINE DYE LABELING REAGENTS FOR SULFHYDRYL GROUPS' CYTOMETRY, ALAN LISS, NEW YORK, US, vol. 10, no. 1, 1989, pages 3-10, XP000071370 ISSN: 0196-4763

Although it seems that the use of dye sets is an essential feature of the present application (description page 3, first paragraph), this feature is not present in the subject-matter of method claims 8 and 15: these claims indeed only refer to the use of a fluorescent dye. Although it has been indicated that the fluorescent dye is selected from a matched set of fluorescent dyes, there is no information in the claims that sets of dyes are actually used. It seems therefore that claims 8 and 15 do not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

Moreover claims 8-14 attempt to define the subject-matter in terms of the result to be achieved, i.e. "characterised in that all available cysteine residues... are labelled with said dye", which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result, which also leads to a lack of clarity (Article 6 PCT).

In any case, the present application does not appear to meet the criteria of Article 33(1) PCT, because the subject-matter of claims 8-14 as presently formulated is not new in the sense of Article 33(2) PCT in view of the fact that document **D1** discloses the labelling of cysteine with fluorescent cyanine dyes as defined in these claims. As can be seen from D1, Table 2, various dyes are used which can be considered as constituting matched sets of fluorescent dyes in the broadest sense of the present application.

Considering the specific structure of the compounds of formula (I) in the matched sets of fluorescent dyes, the subject-matter of claim 1, 15 (as far as it can be interpreted) and claim 19 does not seem to be anticipated or obvious in view of the available prior art (Article 33(2),(3) PCT).

Set 6

1-(6-{[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]amino}-6-oxohexyl)-2-[(1*E*,3*E*)-3-(3,3-dimethyl(1-sulpho-butyl)-1,3-dihydro-2*H*-indol-2-ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound IX); and
1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(3,3-dimethyl-(1-sulpho-butyl)-1,3-dihydro-2*H*-indol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound X).

8. A method for labelling a mixture of proteins in a sample wherein each of said proteins contains one or more cysteine residues, said method comprising:
i) adding to an aqueous liquid containing said sample a fluorescent dye selected from a matched set of fluorescent dyes wherein each said dye contains a target bonding group that is covalently reactive with said proteins; and
ii) reacting said dye with said proteins so that said dye labels said proteins;
characterised in that all available cysteine residues in said proteins are labelled with said dye.

9. A method according to claim 8 wherein said fluorescent dye is a cyanine dye.

10. A method according to claim 9 wherein said cyanine dye contains a sulphonic acid or sulphonate group.

11. A method according to any of claims 8 to 10 wherein said target bonding group is selected from a maleimido group and an iodoacetamido group.

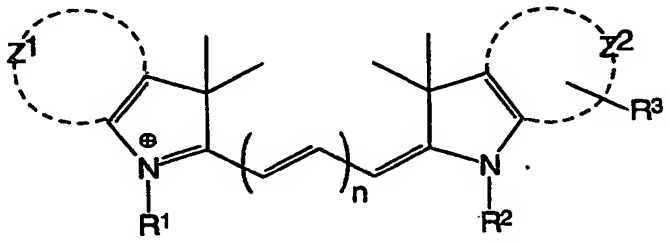
12. A method according to claim 8 further comprising prior to step i), the step of treating the protein with a reductant.

3. A method according to claim 8 wherein said dye is used in a range of 5 to 200nmol of dye per 50µg of protein.

14. A method according to claim 8 wherein said labelling is performed at a pH in the range from 6.0 to 9.0.

15. A method for labelling one or more proteins in a sample, the method comprising:

i) adding to a liquid sample containing said one or more proteins a fluorescent dye selected from a matched set of fluorescent dyes each dye in said set having the formula (I):

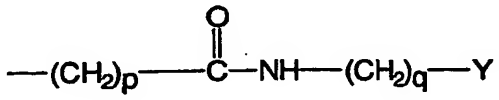


(I)

wherein n is different for each said dye and is 1, 2, or 3;

Z^1 and Z^2 independently represent the carbon atoms necessary to complete a phenyl or naphthyl ring system;

one of groups R^1 and R^2 is the group:



where Y is a target bonding group;

remaining group R^1 or R^2 is selected from $-(CH_2)_4-W$ or $-(CH_2)_r-H$;

group R^3 is hydrogen, except when either R^1 or R^2 is $-(CH_2)_r-H$, in which case R^3 is W;

W is selected from sulphonic acid and sulphonate;

p is an integer from 3 to 6;

q is selected to be 2 or 3; and

r is an integer from 1 to 5;

and their salts;

characterised in that when n of two of said dyes differs by +1, one of p, q and r of said two dyes differs by –1; and

ii) incubating said dye with said sample under conditions suitable for labelling said one or more proteins.

16. A method according to claim 15 wherein each of Z^1 and Z^2 represents the carbon atoms necessary to complete a phenyl ring system.

17. A method according to claim 15 or claim 16 wherein:

n is selected to be 1 or 2;

p is selected to be 4 or 5;

q is selected to be 2 or 3; and

r is selected to be 1, 2 or 3.

18. A method according to any of claims 15 to 17 wherein said target bonding group Y is selected from a maleimido group and an iodoacetamido group.

19. A kit comprising a matched set of fluorescent dyes comprising at least two different fluorescent dyes having the formula (I):